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Essays in Biochemistry: Cell Polarity and Cancer (Guest Editors, Andrew Chalmers and Paul Whitley)

The Hippo pathway: key interaction and catalytic domains in organ growth control, stem cell self-renewal and tissue regeneration

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Abstract

The Hippo pathway is a conserved pathway that interconnects with several other pathways to regulate organ growth, tissue homeostasis and regeneration, and stem cell self-renewal. This pathway is unique in its capacity to orchestrate the multiple processes, from sensing to execution, necessary for organ expansion. Activation of the Hippo pathway core kinase cassette leads to cytoplasmic sequestration of the nuclear effectors YAP and TAZ, consequently disabling their transcriptional coactivation function. Components upstream of the core kinase cassette have not been well understood, especially in vertebrates, but are gradually being elucidated and include cell polarity and cell adhesion proteins. Like many signalling proteins, Hippo pathway proteins are modular and utilise various interaction and catalytic domains to transmit signals and regulate transcription of target genes, often in a context-dependent fashion. In this review we outline the major protein components and focus on the structure and function of some of the key Hippo pathway domains in vertebrates.

Introduction to the Hippo pathway and its role in organ growth control

The Hippo pathway has emerged over the last decade as a key player in organ size regulation during development, tissue homeostasis throughout adult life, tissue regeneration and stem cell self-renewal [1-8]. Perhaps unsurprisingly, the pathway also plays a role in tumour suppression. Hippo pathway components were first identified

through loss of function genetic screens in *Drosophila melanogaster*; the *Hpo* gene, after which the pathway has been called, was named for the mutant overgrown head phenotype that resembled hippopotamus hide [9].

Hippo pathway components

The core elements of the Hippo pathway are well known but additional components constituting an extended network continue to be identified. Core components of the vertebrate pathway (Fig. 1) include the MST1/2 kinases, each of which autophosphorylates its activation loop then phosphorylates and forms an active complex with Sav1. MST1/2 can then phosphorylate the LATS1/2 kinases and their co-activator MOB1. LATS1 or LATS2 subsequently phosphorylates the most downstream targets of the Hippo pathway, YAP and TAZ, enabling 14-3-3 proteins to bind and sequester YAP/TAZ in the cytoplasm (Fig. 1). The Hippo pathway is less complex in *Drosophila* than in vertebrates; where two orthologues exist in vertebrates, for example, only one exists in *Drosophila*. The core components in *Drosophila*, with vertebrate homologues in parentheses, are Hippo (MST1/2), Sav (Sav1), Warts (LATS1/2), MATS (MOB1) and Yorkie (YAP/TAZ).

When the Hippo pathway is inactivated, YAP and TAZ translocate to the nucleus where they behave as co-activators for various transcription factors. YAP and TAZ lack a DNA binding domain and so influence transcription by interacting with DNA binding proteins. Organ growth is promoted by the interaction of YAP and TAZ with TEAD family transcription factors which upregulate transcription of genes that promote cell proliferation, survival, differentiation and morphogenesis [10]. TEAD transcription factors are ubiquitously expressed, though each TEAD protein (TEAD1-TEAD4) occupies a slightly different niche with respect to tissue expression and developmental stage.

Knowledge of upstream signals that regulate organ size and morphology is key to understanding and modulating the Hippo pathway. Recent studies have highlighted the influence of cell contacts on regulation of Hippo and associated pathways. The Crumbs

(Crb) complex, which is associated with tight junctions in the sub-apical region of the cell membrane, regulates the Hippo pathway in *Drosophila* through interactions with the upstream Hippo regulator Expanded (Ex) [11]. It is unclear whether the Crb-Ex mechanism is conserved in mammals but, in response to high cell density, Crb can directly inhibit nuclear translocation of YAP (Fig. 1), in addition to contributing to Hippo pathway activation through an unidentified mechanism [12]. E-cadherin, a trans-membrane protein that forms the intercellular epithelial junction complex, was recently found to be an upstream regulator of the Hippo pathway. E-cadherin induces cell contact inhibition, a phenomenon that stops cellular proliferation upon confluence. E-cadherin recruits to the cell membrane; β -catenin then activates the Hippo pathway through interactions with Merlin/NF2 [13].

Organ growth control and tumourigenesis

Hippo signalling orchestrates organ growth through its coordination of cell proliferation, survival, differentiation and polarity. Numerous examples have illustrated that deregulation of the pathway leads to significantly increased organ size. Upregulation of YAP, the target protein that is inhibited by the Hippo pathway, increased mouse liver size from around 5% of body weight to around 25% in four weeks [14]. Similarly, when core Hippo pathway proteins MST1/2 and Sav1 were knocked out in mouse livers, the organs were significantly larger than those of wild-type mice [15-18]. An enlarged heart phenotype has also been observed in Sav1 knockout mice [19]. In all cases, organ structure was preserved, as observed previously for *Drosophila* Hippo pathway mutants which also display enlarged organs (heads, imaginal discs) with normal tissue patterns [20].

Constitutive YAP over-expression or liver-specific deletion of MST1/2 or Sav1 induces multi-focal tumourigenesis, highlighting the role of the pathway in tumour suppression [14, 16-18]. YAP and TAZ can induce anchorage-independent growth and epithelial-mesenchymal transition (EMT) of immortalised mammary and pancreatic epithelial cells [12]. EMT is important in normal morphological processes but when deregulated in cancers is involved in metastasis, tumour recurrence and therapeutic resistance. Upstream

Hippo pathway components function as tumour suppressors. Hippo pathway mutations have been observed in a range of human cancers including breast cancers, soft tissue sarcomas, melanomas, colorectal cancers, ovarian carcinomas, retinoblastomas, astrocytomas, and neurofibromatosis type 2 [1, 5]. There has been speculation about the possible involvement of Hippo signalling in cancer stem cells due to the pathway's links to stem cell self-renewal and cancer; recently the Hippo pathway, via TAZ, was identified as a molecular link between EMT, cell polarity and cancer stem cells in breast cancer [21].

Integration of multi-pathway signalling

The Hippo pathway interacts with numerous other signalling pathways (Table 1), some of which contribute to organ growth regulation. This cross-talk occurs in both cytoplasm and nucleus and is probably important for the tight control of cell proliferation, growth, polarity and differentiation required for formation and maintenance of functional proportionate organs without crossing the boundary into tumourigenesis. Some of the inter-connections with the Hippo pathway are described below [2, 7, 8, 22].

Canonical Wnt pathway

Wnt signalling activates membrane-bound Dishevelled (Dvl) which inhibits the axin/GSK-3/APC β -catenin destruction complex (β -catenin is the nuclear effector of Wnt signalling). When membrane-localised β -catenin dissociates from E-cadherin and α -catenin, it translocates to the nucleus where it interacts with Lef/Tcf transcription factors, causing the activation of target genes that determine stem cell survival and differentiation. The Hippo pathway inhibits the canonical Wnt pathway by enhancing levels of cytoplasmic phosphorylated TAZ, which binds to Dvl [23]. This interaction prevents Dvl phosphorylation, rendering Dvl inactive. In the absence of hyper-phosphorylated Dvl, β -catenin does not reach the nucleus and is targeted for degradation. The links between Hippo and Wnt signalling are not limited to the cytoplasmic TAZ-Dvl interaction; the E-cadherin/ β -catenin/ α -catenin complex binds Merlin and activates the Hippo pathway [13]. Also, upon Hippo pathway abrogation, nuclear non-phosphorylated TEAD-bound YAP forms a complex with Lef/Tcf-bound β -catenin. YAP- β -catenin

complex formation leads to up-regulation of *Sox2* and *Snai2* genes and consequent increase in heart size [19].

TGF β and BMP signalling

The Hippo pathway has multiple connections with TGF β and BMP signalling. Upon TGF β or BMP interaction with their respective membrane-bound receptors, cytoplasmic Smad proteins are activated by phosphorylation in the C-terminal region. Smad proteins contain an N-terminal MH1 domain that binds DNA and a C-terminal MH2 protein-protein interaction domain. Upon activation, Smads translocate to the nucleus where they form transcription factor complexes. When nuclear Smad2/3 forms a complex with nuclear YAP/TAZ, Smad2/3 is prevented from returning to the cytoplasm and genes that promote EMT are upregulated [12]. Phosphorylation of the Smad inter-MH domain linker by cyclin dependent kinase (CDK) 8/9 promotes Smad-YAP/TAZ interaction which upregulates transcription of target genes when the proteins are nuclear [24]. The Hippo pathway can therefore inhibit TGF β and BMP signalling by inducing cytoplasmic retention of YAP/TAZ, consequently sequestering YAP/TAZ-bound Smad2/3 or Smad1/5/8 proteins to the cytoplasm.

Mechanotransduction

An increase in extracellular matrix (ECM) rigidity, such as in bone, causes Rho GTPase activation and leads to stress fibre formation. When cytoskeletal tension increases in response to stiff ECM, YAP and TAZ are retained within the nucleus and mesenchymal stem cells (MSCs) differentiate into osteoblasts. Conversely, when the MSCs are grown on soft ECM with low intracellular cytoskeletal tension, YAP and TAZ are excluded from the nucleus, and the cells can differentiate into other lineages such as adipocytes. The Hippo pathway regulator E-cadherin can control Rho activation, but in this case E-cadherin may not be involved as mechanotransduction-mediated control of YAP/TAZ cellular localisation occurs independently of the core Hippo pathway [25].

Pro-apoptotic and other interactions

In addition to TEADs and Smads, YAP and TAZ interact with other transcription factors, for example PPAR γ and p73, which can result in repressed transcription or pro-apoptotic effects. YAP and TAZ interact with a host of other proteins (Table 2), in some cases without any apparent connection to the canonical Hippo pathway. As YAP and TAZ are the most downstream targets of the pathway, elucidating the function of these YAP/TAZ complexes is vital for understanding the control of organogenesis and tissue homeostasis.

Key Hippo pathway domains

Regulation of organ growth, tissue homeostasis, tissue regeneration and stem cell self-renewal by Hippo pathway components depends on protein-protein, protein-nucleic acid and protein-membrane interactions and in some instances multi-protein complex formation. The binding properties of a range of domains or motifs within Hippo pathway proteins promote these interactions. Hippo pathway proteins generally contain multiple domains or motifs separated by various lengths of often unstructured polypeptide (Fig. 2).

TEAD-YAP/TAZ interaction domains

The major nuclear proteins regulated by the Hippo pathway are the TEAD transcription factors (Fig. 1). TEAD proteins comprise an N-terminal DNA-binding domain (DBD) and a C-terminal YAP-binding domain (YBD), both of which are indispensable because individually YAP and TEAD have no transcriptional activity. The TEAD DBD can bind to a variety of M-CAT-like DNA sequences (the M-CAT motif is 5'-TCATTCCT-3') in a fairly promiscuous manner. The solution structure of human TEAD1 (TEF-1) A49S mutant DBD comprises a three-helix bundle in a homeodomain-like fold. The first two α -helices are almost anti-parallel with the third α -helix lying across them (Fig. 3). Similar to homeodomain proteins, DNA binding is mediated by the third α -helix (H3) and the preceding loop (L2); H1 and L1 do not bind directly but are necessary for full strength binding of TEAD to tandem M-CAT sites [26].

The TEAD-binding domain (TBD) of YAP/TAZ, located in the N-terminal region, is natively unstructured and binds TEAD with high fidelity - to date no other protein interactions are known to involve the TBD. The crystal structures of YAP-TEAD

interaction domains involving human TEAD2 [27], human TEAD1-YAP [28] and mouse TEAD4-YAP [29] show that the TEAD YBDs adopt an immunoglobulin-like β -sandwich fold with the addition of two helix-turn-helix motifs (Fig. 3). The Y421H mutation in TEAD1 that is present in human Sveinsson's chorioretinal atrophy was previously found to abrogate interactions with YAP and TAZ [30]. Consistent with this, Y421 is located in the TEAD-YAP interface where it forms a hydrogen bond with a Ser residue in YAP (S94 in human YAP). Upon interaction with TEAD, YAP TBD forms two α -helices that pack into binding grooves and are separated by an extended loop that wraps around the YBD [28, 29].

YAP/TAZ transcription activation domain (TAD)

Both YAP and TAZ contain a C-terminal TAD [31]. Although there have been no experimental studies of the structure of this domain, secondary structure predictions indicate that it is largely unstructured.

YAP/TAZ cytoplasmic sequestration by 14-3-3

C-terminal to the YAP/TAZ TBD is an HxRxxS motif that becomes a 14-3-3 binding site upon Ser phosphorylation (S127 in human YAP). A crystal structure of the homodimeric 14-3-3 σ :YAP phospho-peptide complex (Fig. 3) reveals that the YAP peptide binds to each monomer of 14-3-3 σ with 1:1 stoichiometry, so each 14-3-3 dimer can bind two molecules of YAP [32]. 14-3-3 σ dimerises in a W-shape via α -helices 1-4. Helices 3, 5, 7, and 9 on each monomer form the YAP-binding groove. The YAP peptide-bound 14-3-3 σ structure is very similar to unbound 14-3-3 σ with an overall r.m.s.d. (root mean square deviation) between the structures of 1.00 Å, suggesting that YAP binding does not induce a large conformational change in 14-3-3.

WW domains

WW domains are prevalent and important features of the Hippo pathway: YAP, TAZ, Sav1, Kibra and Itch each contain at least one WW domain [33]. The WW domain (approximately 40 residues) is the smallest known protein domain and consists of a twisted three-stranded β -sheet (Fig. 3). WW domains are named after two signature Trp

residues located on the first and third β -strands. The first Trp is required for folding and the second Trp is involved in ligand binding [34]. WW domains are central mediators of protein binding events throughout the extended Hippo network via interactions with proline-rich motifs. WW domains are categorised into five groups (I-V) according to their cognate ligand. The main Hippo pathway WW domains fall into group I i.e. the WW domains bind to PY motifs (PPxY and, less frequently, LPxY) such motifs are found, for example, in LATS1/2, most of the Smads, Dvl, and p73.

Itch contains four WW domains and inhibits the Hippo pathway by binding to PPxY motifs of LATS1/2, predominantly via its first WW domain, leading to ubiquitination and degradation of LATS1/2 [35]. Sav1 and Kibra both contain two WW domains; in each case, WW1 is a group I domain, and WW2 is atypical in that the second Trp is replaced by another amino acid (I in Kibra, Y in vertebrate Sav1 and R in *Drosophila* Sav). Mouse Sav1 WW2 is the only WW domain known to date to dimerise (Fig. 3) [36]. Sav1 promotes multi-protein complex formation in the Hippo pathway by acting as a scaffold protein through SARA domain multimerisation. It is possible that WW2 homodimerisation enhances this scaffolding function whilst WW1 engages binding partners.

The YAP1 and YAP2 isoforms of YAP contain one and two WW domains respectively. It is not currently clear what specific roles the different isoforms play. YAP and TAZ have around 20 known binding partners (Table 2) many of which bind via at least one of the WW domains; given the prevalence of PPxY motifs in proteomes, the number of protein-protein interactions mediated by YAP and TAZ could be much higher than this. The WW domains of YAP and TAZ belong to group I [37], but YAP WW1 has also been found to interact with a phospho-serine motif of Smad1 [24]: phosphorylation by CDK8/9 (as part of BMP signalling) creates a YAP WW1 binding site on the Smad1 inter-MH domain linker (see “*TGF β and BMP signalling*” above). This region also contains a PPxY motif that binds to YAP WW2. CDK8/9 phosphorylation also primes Smad1 for phosphorylation by GSK3. Secondary phosphorylation of Smad1 by GSK3 reduces the affinity for YAP WW1 and increases the affinity for Smurf1 WW1; Smurf1

WW2 simultaneously binds the PPxY motif. Interactions with Smurf1 lead to poly-ubiquitination and subsequent proteasomal degradation of Smad1, thereby marking the end of a YAP-Smad transcriptional event. Wnt signalling suppresses GSK3, providing another illustration of the complexity of inter-pathway connections.

SARAH domains

The SARAH coiled coil domain is present in the C-terminal region of Sav1, Rassf and Hippo (MST1/2). SARAH domains homodimerise and can also mediate heterodimerisation of Hippo pathway proteins, for example between MST1/2 and Sav1. The solution structure of human MST1 SARAH homodimer (Fig. 3) shows that each monomer comprises a short N-terminal α -helix that is oriented towards the N-terminal helix of the other monomer, and an elongated C-terminal α -helix along which the anti-parallel dimer interface lies [38]. The Rassf5 (Nore1) SARAH domain forms a homotetramer but in the presence of MST1 SARAH domain only dimers are observed. The role of mammalian Rassf proteins in the Hippo pathway is currently unclear; *in vitro* studies indicate that Rassf1 and Rassf5 inhibit the Hippo pathway, as is the case for *Drosophila* Rassf. Conversely, in some cases the Hippo pathway seems to be activated by Rassf proteins *in vivo* [1].

Ser/Thr kinase domains

Central to the Hippo pathway are the Ser/Thr kinase domains of MST1/2 and LATS1/2 that propagate phosphorylation events to retain YAP/TAZ in the cytoplasm. MST1/2 belong to the Ste group of kinases, whereas LATS kinases are similar to the PKC family. In a crystal structure of activated MST1 (Fig. 3), the auto-activation loop is di-phosphorylated. There are currently no published structures of the LATS1 and LATS2 kinase domains.

MOB1

MOB1 is part of the Mps One binder (MOB) family of co-activator proteins [39]. Human MOB1 binding to LATS1/2 triggers LATS1/2 auto-phosphorylation on the activation segment. The MOB1-LATS1/2 complex phosphorylates YAP/TAZ. The C-terminal core

domain adopts an α -helical fold common to all MOB proteins whereas the N-terminal region is less conserved but seems to be functionally important. In *S. cerevisiae* MOB1, this N-terminal region includes structural elements that mediate homodimerisation *in vitro* [40]. One side of the MOB1 surface is mostly acidic and the opposite side is basic. Bioinformatic and experimental analyses indicate that the interaction between MOB1 and LATS1/2 is mediated by the acidic face of the former and the basic region of the N-terminal regulatory domain of the latter.

PDZ binding motifs

YAP and TAZ contain a C-terminal PDZ binding motif (LTWL) that allows interaction with several proteins involved in organ size regulation. PDZ domains typically comprise 80-100 residues forming six β -strands, and two α -helices of differing lengths (Fig. 3). The binding groove is generally located between the longer α -helix and the second β -strand [41]. Nuclear localization of YAP/TAZ is promoted by interactions with the first PDZ domain of the tight junction-associated proteins Zonula Occludens-1 and -2 [42, 43]. Interactions involving PDZ binding motifs and PDZ domains, and WW domains and PPxY motifs, are important for Hippo pathway cross-talk with TGF β signalling (YAP/TAZ interaction with Crumbs components PALS1, AMOT, PATJ and LIN7 [12]) and with the Wnt pathway (TAZ interaction with Dvl [23]).

Other domains within Hippo pathway proteins

Remaining protein-protein interaction domains include those involved in self-association such as the dimerisation domain of α -catenin and those that lead to proteasomal degradation e.g. the ubiquitin-associated (UBA) domain of LATS1/2. Domains involved in membrane interaction and localisation include the FERM domains in Merlin and FRMD6, and the C2 domains in Kibra and Itch.

Conclusion

The Hippo pathway inter-connects with numerous other pathways in order to orchestrate organ growth or tissue regeneration, and might therefore be more appropriately termed the Hippo network. Substantial knowledge of Hippo network operation has rapidly

emerged but many questions remain. In terms of protein domains, for example, how are the multiple possible WW, SARAH and PDZ domain interactions coordinated? What are the structural and functional relationships between multiple domains/motifs within and between proteins? How do post-translational modifications, predominantly phosphorylation, modulate domain structures and interactions? Detailed structural, biochemical, biophysical and computational analyses, including isolation or reconstitution of multimolecular complexes, are needed to answer questions such as these. In combination with cellular and organismal studies, one long-term goal of these molecular level studies is systems level comprehension of Hippo signalling towards understanding and prediction of responses to particular developmental and environmental cues, and towards controlled modulation for research and clinical applications.

Summary

- The Hippo pathway is a central, conserved pathway that interconnects with several other pathways to regulate organ growth, tissue homeostasis and regeneration, and stem cell self-renewal.
- The Hippo pathway is unique in its capacity to orchestrate the multiple processes, from sensing to execution, necessary for organ expansion.
- The mechanisms and effects of Hippo pathway cross-talk with other pathways such as Wnt and TGF β growth factor pathways will undoubtedly turn out to be highly complex but are gradually being elucidated.
- The Hippo pathway includes protein domains involved in catalysis, protein-membrane interaction, protein-protein interaction, and protein-nucleic acid interaction.

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Key research papers and recommendations for further reading:

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Figure legends

Figure 1: Mammalian Hippo pathway. The core Hippo pathway components within the orange-bordered box (MST1/2 kinases, scaffolding protein Sav1, LATS1/2 kinases and their cofactors MOB1A/B) target transcriptional co-activator proteins YAP and TAZ for phosphorylation. Phosphorylated YAP and TAZ are subsequently anchored in the cytoplasm by 14-3-3 proteins and the interaction is stabilised by α -catenin. Molecules coloured in grey antagonise the Hippo pathway by inhibiting the core kinases. Inactivation of the Hippo pathway allows YAP and TAZ to translocate into the nucleus where they contribute to the upregulation of target genes through interactions with transcription factors.

Figure 2: Schematic representations of Hippo network proteins.

DBD – DNA binding domain; YBD – YAP binding domain; TBD – TEAD binding domain; WW – domain containing two signature Trp residues; TAD – transactivation domain; M – vinculin-like domain; UBA – ubiquitin-associated domain; S/T KD – Ser/Thr kinase domain; SARA – Sav/Rassf/Hippo domain; FERM – 4.1 protein/ezrin/radixin/moesin domain; CTD – C-terminal domain; PH – pleckstrin homology domain; PDZ - post synaptic density protein/*Drosophila* disc large tumor suppressor/zonula occludens domain; SH3 – SRC homology 3 domain; P-loop – NTPase domain; ECD – extracellular cadherin domain; CCD – cytoplasmic cadherin domain; ARM – armadillo repeat; HECTc - homologous to the E6-AP carboxyl terminus; RA – Ras association domain.

Figure 3: Structures of some Hippo pathway protein domains. A, D, F and H are NMR solution structures; B, C, E, G and I are crystal structures. (A) TEAD1 DBD (PDB ID: 2HZD); (B) TEAD1 YBD:YAP TBD complex (PDB ID: 3KYS); (C) 14-3-3 σ :YAP phospho-peptide complex (PDB ID: 3MHR); (D) YAP WW2 domain (PDB ID: 2L4J); (E) *S. cerevisiae* MOB1 (PDB ID: 2HJN) (F) mouse Sav WW2 domain dimer (PDB ID: 2DWV); (G) ZO1 PDZ1 domain (PDB ID: 2H3M); (H) MST1 SARA domain (PDB ID: 2JO8); (I) Mst1 kinase domain (PDB ID: 3COM).

Table 1 Cross-talk between Hippo pathway proteins and other signalling pathways

Pathway cross-talk	Hippo pathway protein interaction
The Hippo pathway inhibits Wnt/β-catenin signalling (Varelas et al. 2010)	Direct interaction of TAZ with Dvl2 inhibits phosphorylation of Dvl2 by CK1 δ/ϵ thereby preventing formation of the β -catenin destruction complex. This results in β -catenin nuclear localisation and expression of Wnt target genes.
The Hippo pathway inhibits BMP/TGFβ signalling (Alarcon et al. 2009; Varelas et al. 2010)	Direct interaction of YAP with phospho-Smad1 and YAP/TAZ with phospho-Smad2/3 retains Smads in the nucleus and leads to transcription of TGF β /Smad target genes.
The Hippo pathway inhibits JAK/STAT signalling (Karpowicz et al. 2010)	Nuclear Yki induces transcription of cytokines that promote JAK/STAT signalling in response to injury.
The Hippo pathway inhibits Notch signalling (Reddy et al. 2010)	Nuclear Yki inhibits the Notch ligand Delta which leads to Notch activation.
Sonic hedgehog (Shh) signalling inhibits the Hippo pathway (Fernandez et al. 2009)	Shh signalling up-regulates expression of YAP1 mRNA, and stabilises IRS1 (insulin receptor substrate 1) which acts as a nuclear retention factor for YAP. Shh signalling also results in decreased levels of phospho-LATS.
The Hippo pathway promotes FoxO signalling (Choi et al. 2009)	MST1 phosphorylates FoxO proteins leading to nuclear localisation and transcription of FoxO target genes.
PI3 kinase (PI3K)/Akt signalling inhibits the Hippo pathway (Yuan et al. 2010)	Akt phosphorylates MST1 (at T120), thereby preventing the kinase activity of MST1.
Retinoblastoma (Rb) (Nicolay et al. 2011; Tschop et al. 2011)	The transcription factor E2F is negatively regulated by Rb. E2F interacts with the Yki-Sd complex and therefore Rb inhibits E2F-Yki-Sd mediated transcription. In humans, LATS phosphorylates DYRK (dual-specificity tyrosine phosphorylation-regulated kinases) which leads to activation of the 'DREAM' complex and inhibition of E2F.
EGF-Receptor signalling (Zhang et al. 2009)	YAP mediates transcription of the EGF-R ligand amphiregulin, leading to proliferation and migration of neighbouring cells.

Table 2 Binding partners of YAP and TAZ

Binding protein	Description of binding protein	YAP/TAZ domain involved
14-3-3 (Kanai et al. 2000; Basu et al. 2003)	Dimeric proteins that sequester YAP/TAZ to the cytoplasm	Phosphorylated 14-3-3 binding motif
AMOT (Chan et al. 2011)	Angiomotin part of the Crumbs complex	WW domain(s)
ASPP1/2 (p53BP) (Espanel and Sudol 2001; Liu et al. 2011)	Apoptosis-stimulating protein of p53 protein family	WW domain(s)
c-Yes (Sudol et al. 1995)	Tyrosine kinase	SH3 binding motif
Crb (Varelas et al. 2010)	Upstream Hippo pathway complex protein Crumbs	WW domain(s) and PDZ-binding motif
Dvl2 (Varelas et al. 2010)	Dishevelled polarity protein involved in Wnt signalling	WW domain and PDZ binding motif
ErbB4 (Komuro et al. 2003)	Receptor tyrosine kinase that contains a cleavable cytoplasmic fragment	WW domain(s)
Ex (Badouel et al. 2009)	Upstream Hippo pathway FERM-domain protein	WW domain(s)
HNRNPU (Howell et al. 2004)	Heterogenous nuclear ribonuclear protein U binds to YAP and p73	Proline-rich region at N-terminus of YAP
LATS1/2 (Lei et al. 2008; Zhang et al. 2008)	Hippo pathway Ser/Thr kinases	WW domain(s)
NFE2 (p45) (Gavva et al. 1997)	Haemopoietic transcription factor-2	WW domain(s)
NHERF (EPB50) (Mohler et al. 1999; Kanai et al. 2000)	Recruits YAP and TAZ to plasma membrane	PDZ binding motif
p73 (Strano et al. 2001; Oka and Sudol 2009)	Pro-apoptotic transcription factor	WW domain(s)
PEBP2 (Yagi et al. 1999)	Polyoma enhancer binding protein 2 transcription factor	WW domain(s)
PPARγ (Hong et al. 2005)	Adipocyte transcription factor	WW domain(s)
PRGP2 (Kulman et al. 2007)	Proline-rich membrane Gla protein	WW domain(s)
Runx1/2 (Zaidi et al. 2004; Hong et al. 2005)	Transcription factors with Runt DNA-binding domain	WW domain(s)
Smads (Alarcon et al. 2009; Aragon et al. 2011)	Transcription factors regulated by the TGF β BMP signalling pathway	WW domain(s)
TEAD (Vassilev et al. 2001)	Transcription factors	TBD
WBP1/2 (Chen and Sudol 1995)	WW domain-binding proteins	WW domain(s)

Biographical note for authors

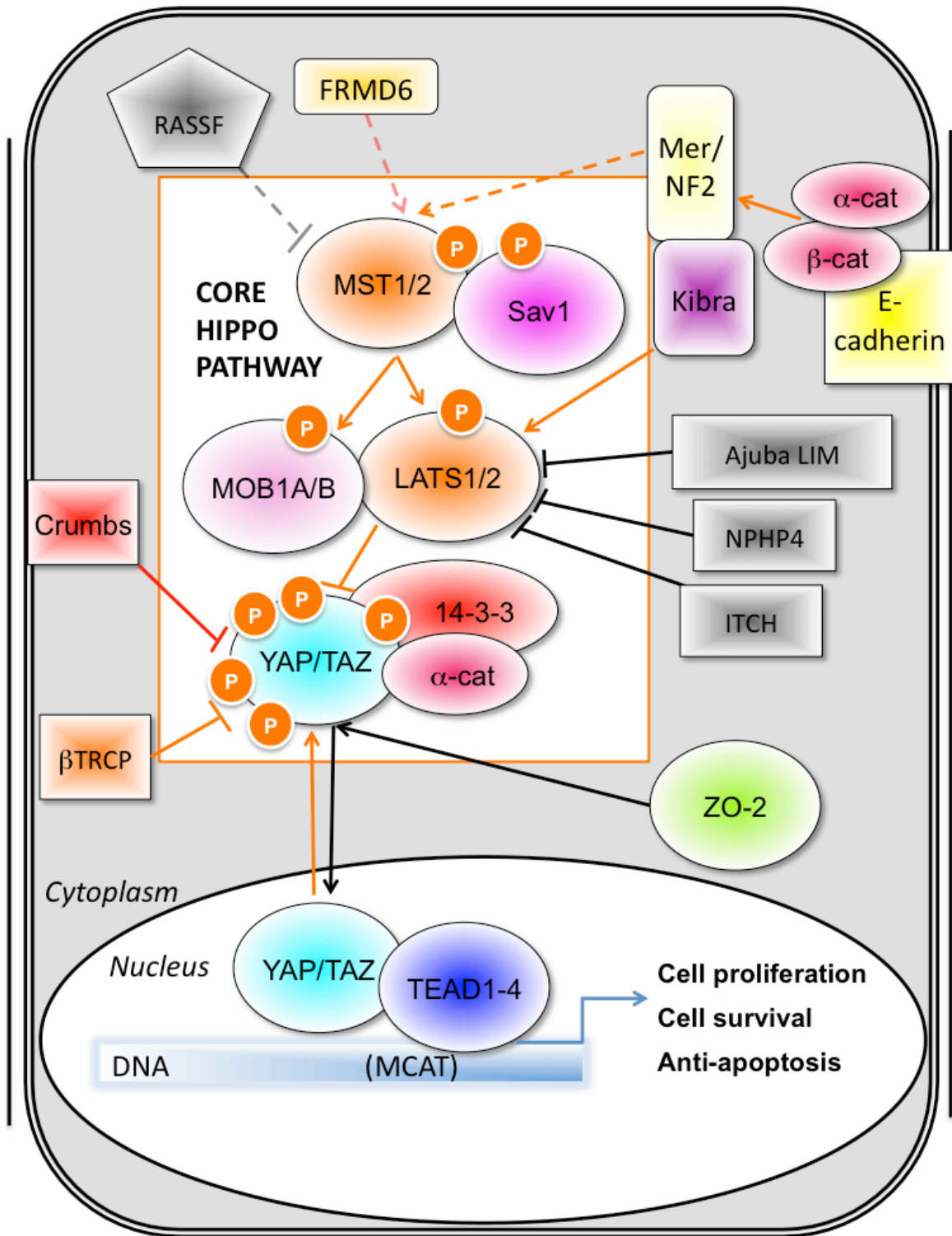
Claire Cherrett (née Webb) has just completed her PhD at University of Bath, working with Stefan Bagby, Jean van den Elsen and Makoto Furutani-Seiki. Claire worked on structure and function of Hippo pathway proteins using both NMR spectroscopy and X-ray crystallography.

Makoto Furutani-Seiki has been at University of Bath since 2007. Makoto holds an MRC Senior Non-Clinical Fellowship. He previously did MD and PhD degrees in Japan, post-doctoral work with Christiane Nüsslein-Volhard in Tübingen, and held group leader positions in Germany and Japan where he was involved with large scale mutagenesis screens in both zebrafish and medaka fish. His group uses medaka fish as a model organism to study the cellular and molecular mechanisms of organ growth control.

Stefan Bagby has been at University of Bath since 1999. Before that, he did a DPhil with Allen Hill in Oxford, followed by post-doctoral work with Mitsu Ikura in Toronto on structure and function of transcription factors and calcium binding proteins. His group has been studying WW and C2 domains of Hippo pathway proteins.

Key words for index

Canonical Wnt, EMT, FERM domain, 14-3-3, Hippo pathway, Kibra, LATS1/2 kinase, MOB1, MST1/2 kinase, PDZ domain, SARAH domain, Sav1, TAZ, TEAD, TGF β , WW domain, YAP



TEAD 1-4

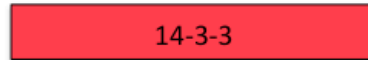
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**YAP/TAZ**

(~470 aa)

**14-3-3**

(~250 aa)

 **α -catenin**

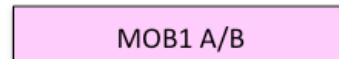
(~900 aa)

**LATS1/2**

(~800-1000 aa)

**MOB1A/B**

(~220 aa)

**Sav1**

(~380 aa)

**Mst1/2**

(~480 aa)

**Kibra**

(~1110 aa)

**Merlin**

(~550 aa)

**Ex/FRMD6**

(~1400 or 600 aa)

**ZO1/2**

(~1100-1750 aa)

**E-cadherin**

(~880 aa)

 **β -catenin**

(~780 aa)

**Itch**

(~900 aa)

**RASSF1A**

(~340 aa)



